

Paroxysmal nocturnal hemoglobinuria

Paroxysmal nocturnal hemoglobinuria (PNH), previously **Marchiafava–Micheli syndrome**, is a rare, acquired,^[1] life-threatening disease of the blood characterized by **destruction of red blood cells** by the **complement system**, a part of the body's intrinsic immune system. This destructive process is a result of a defect in the formation of surface proteins on the red blood cell, which normally function to inhibit such immune reactions. Since the complement cascade attacks the red blood cells throughout the circulatory system, the hemolysis is considered an *intravascular* hemolytic anemia. Other key features of the disease, notably the high incidence of **thrombosis**, are not totally understood.^[2]

PNH is the only hemolytic anemia caused by an *acquired* (rather than inherited) intrinsic defect in the **cell membrane** (deficiency of **glycophosphatidylinositol** leading to absence of protective proteins on the membrane).^[3] It may develop on its own ("primary PNH") or in the context of other **bone marrow** disorders such as **aplastic anemia** ("secondary PNH"). Only a minority (26%) have the telltale red urine in the morning that originally gave the condition its name.^[4]

Allogeneic bone marrow transplantation is the only curative therapy, but has significant rates of both mortality and ongoing morbidity.^[5] The monoclonal antibody eculizumab is effective at reducing the need for blood transfusions and improving quality of life.^[5] It, however, does not appear to change the risk of death or blood clots and has an extremely high price, with a single year of treatment costing at least \$440,000.^{[6][7]}

1 Signs and symptoms

The classic sign of PNH is **red discoloration of the urine due to the presence of hemoglobin** and **hemosiderin** from the breakdown of **red blood cells**. As the urine is more concentrated in the morning, this is when the color is most pronounced. This phenomenon mainly occurs in those who have the primary form of PNH, who will notice this at some point in their disease course. The remainder mainly experience the symptoms of anemia, such as **tiredness**, **shortness of breath**, and **palpitations**.^[4]

A small proportion of patients report attacks of **abdominal pain**, **difficulty swallowing** and **pain during swallowing**, as well as **erectile dysfunction** in men; this occurs mainly when the breakdown of red blood cells is rapid, and is attributable to spasm of **smooth muscle** due to red cell breakdown products.^[4]

Forty percent of people with PNH develop **thrombosis** (a blood clot) at some point in their illness. This is the main cause of severe complications and death in PNH. These may develop in common sites (**deep vein thrombosis** of the leg and resultant **pulmonary embolism** when these clots break off and enter the lungs), but in PNH blood clots may also form in more unusual sites: the **hepatic vein** (causing **Budd-Chiari syndrome**), the **portal vein** of the liver (causing **portal vein thrombosis**), the **superior or inferior mesenteric vein** (causing **mesenteric ischemia**) and veins of the skin. **Cerebral venous thrombosis**, an uncommon form of **stroke**, is more common in those with PNH.^[4]

2 Diagnosis

Blood tests in PNH show changes consistent with **intravascular hemolytic anemia**: **low hemoglobin**, **raised lactate dehydrogenase**, **raised bilirubin** (a breakdown product of hemoglobin), and decreased levels of **haptoglobin**; there can be raised **reticulocytes** (immature red cells released by the **bone marrow** to replace the destroyed cells) if there is no **iron deficiency** present. The **direct antiglobulin test** (DAT, or direct Coombs' test) is negative, as the **hemolysis** of PNH is not caused by **antibodies**.^[4] If the PNH occurs in the setting of known (or suspected) **aplastic anemia**, abnormal **white blood cell** counts and decreased **platelet** counts may be seen at this. In this case, anemia may be caused by insufficient red blood cell production in addition to the hemolysis.^[4]

Historically, the sucrose lysis test, in which a patient's red blood cells are placed in low-ionic-strength solution and observed for hemolysis, was used for screening. If this was positive, the *Ham's acid hemolysis test* (after Dr Thomas Ham, who described the test in 1937) was performed for confirmation.^{[5][8]}

Today, the gold standard is **flow cytometry** for **CD55** and **CD59** on **white** and **red blood cells**. Based on the levels of these cell proteins, erythrocytes may be classified as type I, II, or III PNH cells. Type I cells have normal levels of CD55 and CD59; type II have reduced levels; and type III have absent levels.^[4] The **fluorescein-labeled proaerolysin (FLAER)** test is being used more frequently to diagnose PNH. FLAER binds selectively to the glycosphosphatidylinositol anchor and is more accurate in demonstrating a deficit than simply for CD59 or CD55.^[5]

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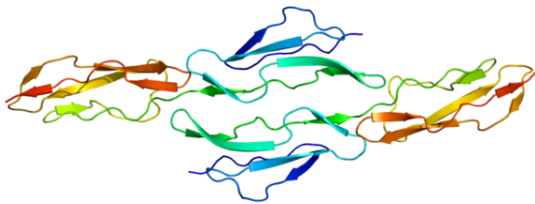
SCREENING

2.1 Classification

PNH is classified by the context under which it is diagnosed:^[4]

- *Classic PNH*. Evidence of PNH in the absence of another bone marrow disorder.
- *PNH in the setting of another specified bone marrow disorder* such as aplastic anemia and myelodysplastic syndrome (MDS).
- *Subclinical PNH*. PNH abnormalities on flow cytometry without signs of hemolysis.

3 Pathophysiology



CD55 protein/Decay Accelerating Factor structure



CD59 protein/Protectin structure

All cells have proteins attached to their membranes, often serving as a mode of communication or signaling between the cell and the surrounding extracellular milieu. These signaling proteins are physically attached to the cell membrane in various ways, commonly anchored by glycolipids such as **glycosyl phosphatidylinositols (GPI)**. PNH occurs as a result of a defect in the assembling of these glycolipid-protein structures on the surface of blood cells.^[4]

The most common defective enzyme in PNH is **phosphatidylinositol glycan A (PIGA)**, one of several enzymes needed to make GPI. The gene that codes for PIGA is located on the **X chromosome**, which means that only one active copy of the gene for PIGA is present in each cell (initially, females have two copies, but one is silenced through **X-inactivation**).^[1] A mutation in the PIGA gene can lead to the absence of GPI anchors expressed on the cell membrane. When this mutation occurs in a **hematopoietic stem cell** in the bone marrow, all of the cells it produces will also have the defect.^[4]

Several of the proteins that anchor to GPI on the cell membrane are used to protect the cell from destruction by the **complement system**, and, without these anchors, the cells are more easily targeted by the complement proteins.^[3] Although red blood cells, white blood cells and platelets are targeted by complement, red blood cells are particularly vulnerable to lysis. The complement system is part of the **innate immune system** and has a variety of functions, from destroying invading microorganisms by **opsonization** to direct destabilization by the **membrane attack complex**. The main proteins that protect blood cells from destruction are **decay-accelerating factor (DAF/CD55)**, which disrupts formation of **C3-convertase**, and **protectin (CD59/MIRL/MAC-IP)**, which binds the **membrane attack complex** and prevents **C9** from binding to the cell.^[4]

The symptoms of **esophageal spasm**, erectile dysfunction, and abdominal pain are attributed to the fact that **hemoglobin** released during hemolysis binds with circulating **nitric oxide**, a substance that is needed to relax **smooth muscle**. This theory is supported by the fact that these symptoms improve on administration of nitrates or **sildenafil (Viagra)**, which improves the effect of nitric oxide on muscle cells.^[4] There is a suspicion that chronic hemolysis causing chronically depleted nitric oxide may lead to the development of **pulmonary hypertension** (increased pressure in the blood vessels supplying the lung), which in turn puts strain on the **heart** and causes **heart failure**.^[9]

Historically, the role of the sleep and night in this disease (the “nocturnal” component of the name) has been attributed to acidification of the blood at night due to relative **hypoventilation** and accumulation of carbon dioxide in the blood during sleep. This hypothesis has been questioned by researchers who note that not all those with PNH have increased hemolysis during sleep, so it is uncertain how important a role sleep actually plays in this disease.^[10]

4 Screening

There are several groups where screening for PNH should be undertaken. These include patients with unexplained thrombosis who are young, have thrombosis in an unusual site (e.g. intra-abdominal veins, cerebral veins, dermal veins), have any evidence of hemolysis (i.e. a raised LDH), or have a low red blood cell, white blood cell, or platelet count.^[11] Those who have a diagnosis of aplastic anemia should be screened annually.^[4]

5 Treatment

5.1 Acute attacks

There is disagreement as to whether **steroids** (such as **prednisolone**) can decrease the severity of hemolytic crises. Transfusion therapy may be needed; in addition to correcting significant **anemia**, this suppresses the production of PNH cells by the bone marrow, and indirectly the severity of the hemolysis. Iron deficiency develops with time, due to losses in urine, and may have to be treated if present. Iron therapy can result in more hemolysis as more PNH cells are produced.^[4]

5.2 Long-term

PNH is a chronic condition. In patients with only a small clone and few problems, monitoring of the flow cytometry every six months gives information on the severity and risk of potential complications. Given the high risk of thrombosis in PNH, preventive treatment with **warfarin** decreases the risk of thrombosis in those with a large clone (50% of white blood cells type III).^{[4][12]}

Episodes of thrombosis are treated as they would in other patients, but, given that PNH is a persisting underlying cause, it is likely that treatment with **warfarin** or similar drugs needs to be continued long-term after an episode of thrombosis.^[4]

5.3 Eculizumab

In 2007, the drug **eculizumab** was approved for the treatment of PNH. It improves quality of life and decreases the need for blood transfusions but does not appear to affect the risk of death.^[7] It does not appear to change the risk of blood clots, **myelodysplastic syndrome**, **acute myelogenous leukemia**, or **aplastic anemia**.^[7]

Eculizumab is controversial due to its high cost as it is among the most expensive pharmaceuticals in the world, with a price of US\$440,000 per person per year.^[13]

6 Epidemiology

PNH is rare, with an annual rate of 1-2 cases per million.^[4] The prognosis without disease-modifying treatment is 10–20 years.^[14] Many cases develop in people who have previously been diagnosed with aplastic anemia or **myelodysplastic syndrome**. The fact that PNH develops in MDS also explains why there appears to be a higher rate of **leukemia** in PNH, as MDS can sometimes transform into leukemia.^[4]

25% of female cases of PNH are discovered during pregnancy. This group has a high rate of thrombosis, and the

risk of death of both mother and child are significantly increased (20% and 8% respectively).^[4]

7 History

The first description of paroxysmal hemoglobinuria was by the **German** physician Paul Strübing (**Greifswald**, 1852–1915) during a lecture in 1881, later published in 1882.^[15] Later comprehensive descriptions were made by Ettore Marchiafava and Alessio Nazari in 1911,^[16] with further elaborations by Marchiafava in 1928^[17] and Ferdinando Micheli in 1931.^{[18][19]} The Dutch physician Enneking coined the term “paroxysmal nocturnal hemoglobinuria” (or *haemoglobinuria paroxysmalis nocturna* in Latin) in 1928, which has since become the default description.^[20]